benefits paid under the federal laws. About 3,700 infant health centers were maintained with public funds, and they reached about two-thirds of the 700,000 children born annually in Prussia. A larger proportion of children are cared for at the centers in the cities than in the rural districts.

In many cities the work of the infant health centers extends to children of preschool age; in other cities special centers are provided for such children. About 400,000 children were reached by the centers reporting to the Ministry of Social Welfare. The public authorities also maintain large numbers of kindergartens and other arrangements for children of preschool age, such as day centers, rest homes in cities and in the country, clinics for ultra-violet ray treatments, and lunch rooms.

According to the report the health work extends to a larger proportion of school children—3,000,000 out of 3,600,000—than to children of other ages. Almost 3,200 school physicians and 4,600 school nurses were employed in the last year. Dental service has been given in the great majority of public schools; of the nearly 2,000,000 children whose teeth were examined in 1929, 44 per cent were found to be in need of treatment. School lunches were served to half a million children in about one-fifth of the public schools in Prussia. The municipal authorities are also maintaining a large number of rest homes and vacation homes for school children, and more than 600,000 children were thus accommodated in 1929.—*Archiv. f. Soziale Hygiene und Demographie*, Berlin, Vol. 6 (Sept.), 1931.

LABORATORY

A MODIFIED CELLOBIOSE BROTH FOR THE DIFFERENTIATION OF B. COLI AND B. AEROGENES

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CELLOBIOSE was first proposed as a medium for the differentiation of *B. coli* and *B. aerogenes* by Jones and Wise[1] in 1926, and was very carefully correlated with other mediums by Koser.[2] It was later used by Lewis and Pittman[3] and by Perry[4] and probably others.

The authors of the method, Jones and Wise, used this sugar in a concentration of 0.5 per cent in a broth containing 0.3 per cent Difco beef extract and 1 per cent Difco peptone. It was sterilized in an autoclave for 20 minutes at approximately 121° C. They do not tell how acid production was determined.

Koser, in his study of the method, added 0.5 per cent of cellobiose to a standard meat extract, peptone medium, plus 0.5 per cent agar, plus bromthymol-blue (pH range 6.0 to 7.6) in the amount suggested by Baker[5] (12 c.c. of a 0.2 per cent alcohol solution per liter). It was put into small tubes, in 2 to 3 c.c. amounts, and sterilized in an autoclave. This made a soft agar which was inoculated by stabbing with a straight wire. Gas production was detected by the torn appearance of the

agar medium and acid formation by the change in the indicator, brom-thymol-blue (blue to yellow). Incubation was at 30° C. for 48 hours as a routine procedure.

Lewis and Pittman employed Koser's method. Perry used the same soft agar method and, to a limited extent, broth fermentation tubes. The concentration of cellobiose used was 0.5 per cent and brom-thymol-blue was employed as an indicator for acid formation. A 4-day incubation at 37° C. was used. He says of it: "The cellobiose test has been found the most satisfactory single test for this purpose" (differentiation of *B. coli* and *B. aerogenes*).

In the method here reported the medium used was a standard sugar-free broth containing 0.3 per cent of beef extract and 0.5 per cent of peptone (Armour's bacteriological). To each 100 c.c. of this broth was added 0.25 gm. of cellobiose (Difco) and for an indicator of acid formation, 0.875 c.c. of a 0.05 per cent solution of brom-cresol purple (pH range 5.2 to 6.8). It was tubed in very small fermentation tubes and sterilized in an Arnold sterilizer, 45 minutes on 3 successive days. (The experience of Jones and Wise and Koser indicates that it can safely be autoclaved.) The regular inner tubes (8 mm. inside diameter) of the Dunham fermentation vials were used as the outer tubes. For inner tubes, glass tubing of about 5 mm. outside diameter was cut into 7/8" lengths and these sealed at one end. One c.c. is sufficient medium for one of these fermentation tubes.

In the first trial of this medium a concentration of 0.5 per cent of cellobiose was used. In a later batch a concentration of 0.25 per cent was accidentally employed and as the lot of medium apparently worked just as well, this concentration was adopted. (It was stated by the authors of the method that a lower concentration of the sugar might prove equally satisfactory.)

TABLE I

CORRELATION OF CELLOBIOSE TEST WITH OTHER MEDIUMS AND METHODS FOR THE DIFFERENTIATION OF B. COLI AND B. AEROGENES

Culture	37° C. Incubation			46° C. Incubation
	Cellobiose Broth		Koser's Citrate Broth	Eijkman's Dextrose Peptone Broth Gas Formation
	Acid Formation	Gas Formation		
Group I—Typical B. Coli from Feces Freshly Isolated				
1. McAllister T_1	−	−	−	5%
2. " T_2	−	−	−	5%
3. Griggs T	−	−	−	10%
4. Abell T_1	−	−	−	10%
5. " T_2	−	−	−	10%
6. Ornsbee T	−	−	−	20%
7. Hoffman T_1	−	−	−	10%
8. " T_2	−	−	−	5%
9. " T_3	−	−	−	−
Group II—Atypical B. Coli not Freshly Isolated				
10. No. 2	−	−	−	20%
11. No. 3	−	−	−	30%
12. No. 4	−	−	−	5%
13. No. 5	−	−	−	10%
Group III—B. Aerogenes				
14. No. 1	+	50%	+	−
15. No. 2	+	10%	+	−
16. No. 3	+	75%	+	−
17. No. 5	+	50%	+	−
18. No. 11	+	50%	+	−
Group IV—Irregular or Intermediate Forms				
19. Sewage E	+	Trace only	+	
20. Sewage F	+	−	−	
21. Feces—Montgomery	−	−	+	

It has been employed many times as a differentiating medium in studying the growths on Levine's Eosin Methylene Blue Agar, transfers being made by means of a straight wire from separate colonies on the plate. Incubation is at 37° C. Acid and gas formation with *B. aerogenes* is rapid, easy to observe, and frequently positive in 24 hours. In 48 hours the results are usually final. In only a very few cases were the results indeterminate, that is, acid + and gas — or acid — and gas +.

It was found to work with great uniformity of results on pure cultures of *B. coli* and *B. aerogenes* freshly isolated from feces. A few correlations were made with Koser's citrate and with Eijkman's dextrose broth at 46° C. incubation. The accompanying table shows a selected list of these results. There are included a few that do not show perfect correlation. They indicate quite definitely that this medium may be depended upon to give results that are in line with those found by Koser and others.

At $6.00 per gm. for cellobiose the cost for this constituent is only $.015 per tube. With a reduction in the price of this sugar in the market, which may reasonably be expected, this obstacle in the way of its more general use would be removed.

SUMMARY

The cellobiose broth of the authors, Jones and Wise, has been modified by the reduction of the concentration of cellobiose to 0.25 per cent and the addition of brom-cresol purple as an indicator. The cost has been reduced by tubing in micro tubes that require only 1 c.c. for filling. Gas and acid formation are rapid and definite and sufficient correlation study has been made to indicate that the same results are obtainable as those found by Koser, who noted excellent correlation in the case of the two well defined sections of the group, with several other differentiating mediums, particularly with citrate broth, and also with the known source of the culture.

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PREPARATION OF MEDIUM FOR THE CULTURE OF BACTERIUM TULARENSE

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LABORATORY workers have experienced some difficulty in growing *B. tularense*. This is especially true when the organism is cultured on a large scale for the preparation of antigen where the addition of blood to the medium is undesirable.

The following method of preparing the medium has proved satisfactory in the laboratory. The ingredients present and the quantities used are those recommended by Francis, but the method of preparation has been modified somewhat.

Beef infusion broth is prepared in the usual manner with the addition of 1 per cent peptone and 0.5 per cent NaCl. This is titrated and the reaction adjusted to pH 7.3. The broth is then sterilized for 15 minutes at 15 lb. pres-

sure, cooled, filtered and titrated again. Any needed correction in reaction is made and the broth again sterilized at 15 lb. for 10 minutes. It is then titrated a third time and the reaction again corrected to pH 7.3 if necessary. With each sterilization the change in reaction becomes progressively less and often no correction is necessary after the third titration.

It was found that subsequent sterilization did not change the reaction and that additional titrations of the medium were therefore unnecessary. It is this drift toward the acid phase which, if left uncorrected, probably accounts for more failures in the culture of *B. tularense* than any other one factor. While this method is suggested for *B. tularense,* it should be equally applicable to any organism that has similar requirements of a definite and constant pH value.

After the third titration and correction, 2 per cent agar, 1 per cent dextrose and 0.1 per cent pulverized cystine are added to the broth. The medium is distributed into large bottles or into test tubes, depending on whether it is to be used for stock cultures or for growing the organism for antigen. The medium is then given a final sterilization at 15 lb. for 20 minutes.

For antigen production we use 1 liter prescription bottles, adding 150 c.c. of the medium to each bottle. For stock cultures rabbit blood is added to the tubes under aseptic conditions.

The agar in the large bottle is agitated just before it is cool enough to solidify. This is desirable, as the cystine, being insoluble, settles out with any precipitate which may form during sterilization. Each bottle is placed on its flat side and allowed to solidify. The flat surface thus exposed is inoculated in the usual way. *B. tularense* grows on this medium with an odor resembling freshly cooked meat. Any sour or musty odor is in itself proof of contamination even before it is confirmed by stained preparations.